PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2003-171290

(43)Date of publication of application : 17.06.2003

(51)Int.Cl. A61K 35/64
A23K 1/16
A23K 1/18
A23L 1/076
A23L 1/30
A23L 1/302
A61K 7/00
A61K 31/341
A61P 17/00

(21)Application number : 2002-201883

(22)Date of filing : 10.07.2002

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(30)Priority

Priority number : 2001295464 Priority date : 27.09.2001 Priority country : JP

(54) METHOD FOR PRODUCING COLLAGEN PRODUCTION POTENTIATOR AND APPLICATION OF THE SAME

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a collagen production potentiator capable of continuously exhibiting action of potentiating the collagen production by using L-ascorbic acids.

SOLUTION: This collagen production potentiator contains the L-ascorbic acids and royal jellies as active ingredients.

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CLAIMS

[Claim(s)]
[Claim 1]A collagen production enhancement agent which contains L-ascorbic acid and royal jelly as an active principle.
[Claim 2]the collagen production enhancement agent according to claim 1 which contains any of a field of an eating-and-drinking article, a food for specified dietary use, a food with health claims, cosmetics, quasi drugs, drugs, feed, a feed, and pet food, one sort set and used, or two sorts or more of other ingredients with L-ascorbic acid and royal jelly.
[Claim 3]The collagen production enhancement agent according to claim 2, wherein other ingredients are one sort chosen from an anti-oxidant, a thickener, sugars, and sugar-alcohol, or two sorts or more.
[Claim 4]The collagen production enhancement agent according to any one of claims 1 to 3 containing L-ascorbic acid 0.02% of the weight or more by weight conversion as L-ascorbic acid.
[Claim 5]When weight conversion is carried out by making L-ascorbic acid into L-ascorbic acid, The collagen production enhancement agent according to any one of claims 1 to 4 characterized by containing 0.5 or more weight sections of royal jelly by weight conversion as non-heat-treating royal jelly to L-ascorbic acid 1 weight section which carried out weight conversion.
[Claim 6]The collagen production enhancement agent according to any one of claims 1 to 5, wherein L-ascorbic acid is L-ascorbic acid 2-glycosides.
[Claim 7]The collagen production enhancement agent according to claim 6, wherein L-ascorbic acid 2-glycoside contains L-ascorbic acid 2-glucoside at least.
[Claim 8]The collagen production enhancement agent according to any one of claims 1 to 7, wherein royal jelly is heat-treated for more than 30 minutes above 70 **.
[Claim 9]A constituent containing the collagen production enhancement agent according to any

one of claims 1 to 8.

[Claim 10]An eating-and-drinking article, a food for specified dietary use, a food with health claims, cosmetics, quasi drugs, drugs, feed, a feed, the constituent according to claim 9 being in any of pet food.

[Claim 11]A manufacturing method of the collagen production enhancement agent according to any one of claims 1 to 8 including a process of blending L-ascorbic acid and royal jelly.

[Claim 12]A TGF-beta production enhancement agent containing royal jelly.

[Claim 13]The TGF-beta production enhancement agent according to claim 12 containing L-ascorbic acid with royal jelly.

[Claim 14]A keratinocyte growth accelerator containing royal jelly.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to the constituent containing a new collagen production enhancement agent, the collagen production enhancement agent which contains L-ascorbic acid and royal jelly in detail, and the collagen production enhancement agent concerned.
[0002]
[Description of the Prior Art]L-ascorbic acid (vitamin C) is one of the indispensable nutrients which are not produced with Homo sapiens, an ape, and a guinea pig in the living body. it is not only known that prevention and the therapy of scurvy have an effect, but L-ascorbic acid is bearing the role important for the health maintenance and improvement of a living body in the living body with regards to many physiological functions, such as an immunoenhancing effect by the collagen production and the leukocytosis which are the main ingredients of connective tissue. L-ascorbic acid does not remain in an essential nutrient, but is only used for the eating-and-drinking article as an acid taste agent, a pH regulator, an antioxidant, a browning inhibitor, etc.
In addition, it is broadly used for general cosmetics, such as lustrous skin agents, such as preventive and a treating agent of various diseases, such as viral illness, bacterial disease, and a malignant tumor disease, and also an ultraviolet ray absorbent, and a melanin generation depressant, and a milky skin agent.
L-ascorbic acid is unstable in order that it may show direct reduction nature, Since it is easy to receive oxidative degradation and the physiology activity decreases easily, L-ascorbic acid derivatives, such as L-ascorbic acid glucoside, L-ascorbic acid phosphoric acid, and L-ascorbic acid sulfuric acid, are developed for stabilization of L-ascorbic acid. However, since each of they is manufactured by the raw material in L-ascorbic acid, the cost cannot but become higher

than L-ascorbic acid inevitably. Since itself is strong acid nature on the other hand when these are applied to a living body in large quantities, even if it uses L-ascorbic acid and/or an L-ascorbic acid derivative, To that temporary obstacle nature to the membrane in the skin, the mouth, and the stomach and intestines and the metabolite of L-ascorbic acid generate in large quantities, and a pan. Considering an economical merit, in the health food taken in every day, also in the state where the amount of the L-ascorbic acid used was reduced, a constituent which the physiological function can fully demonstrate is desired.

[0003]On the other hand, royal jelly is the secrete of the opalescence from a worker's exocrine gland accumulated in the royal cell (fringe of a queen bee) in the nest of a honeybee.

It is the food the larva which should serve as a queen bee is fed.

At the time, although the larva of the honeybee which hatched by the royal cell is undistinguishable from a worker, if royal jelly is fully taken in and it grows, it will grow up to be a queen bee which the body is large compared with a worker, is long-life, and has many egg productions. It was known that it is expected that there are a variety of physiological functions, such as sthenia and strong energy, in royal jelly, it is used more as health food from ancient times, and there are actually these operations experientially from this. Much scientific reports are made in recent years about the antibacterial action of royal jelly, an immunoenhancing effect, antitumor action, anti-inflammatory activity, etc. It is thought that it does not invite critical side effects even if it applies it to animals including Homo sapiens, since royal jelly is a natural product. From these things, royal jelly is widely used as a raw material of health food or cosmetics.

[0004]L-ascorbic acid and royal jelly are widely used as a raw material of health food or cosmetics as above-mentioned.

the ingesta (JP,2000-342331,A.) containing L-ascorbic acid and royal jelly JP,2000-60455,A, skin external preparations (JP,63-03706,A), the skin external preparations (JP,2000-63226,A) which contain an L-ascorbic acid derivative and royal jelly further, etc. are already developed.

[0005]However, there is nothing that noted the point that royal jelly reinforced collagen production, and there is until now. [that has acquired knowledge that royal jelly reinforces the collagen production by ascorbic acid]

[0006]An aging society is greeted and the change of reduction of the thickness of the skin, a fall of metabolism, etc. accompanying aging is a source of a trouble for many middle and old age people of a world, division, and a woman today.

Especially change of the looks accompanying generating of the small JIWA wrinkles of the face sensed remarkable, a stain, and sag, reduction in gloss and disappearance of a beam, reduction of the elasticity of the skin, etc. is the typical thing.

Although the cosmetics which blended collagen and mucopolysaccharides, such as hyaluronic

acid, have so far been developed as cosmetics for aging prevention in order to secure the moistness of the skin, sufficient effect for the aging prevention of the skin is not acquired only by it. When the research on aging progressed in recent years, it has become clear that main a remarkable reduction of the collagen fiber which forms dermis causes aging of the skin. And reduction of small JIWA of the face, wrinkles and a stain, or gloss, disappearance of a beam, generating of sag, Although it is suggested that the cause of change of looks, such as a fall of the elasticity of the skin, is related to reduction in a collagen fiber and some causes of aging of the skin are various, after all, It concludes in the metabolic turnover of collagen falling due to the fall of the collagen production ability of the fibroblast which exists in dermis, and the fall of fibroblast's own fecundity. In order that the epidermis which consists of keratinocytes outside dermis may exist in the skin and the cornified layer of a skin surface may become weak also due to the depression of this keratinocyte, Renewal of a cornified layer is not only overdue, but the fall of the biophylaxis ability which is one of the original functions of the skin is caused, and since the damage to the whole skin increases the fibroblast of dermis from the first, aging of the skin is further promoted by various factors including bacterial infection. The fall of the production amount of collagen in fibroblast causes the tissue of the inside of the body where the structure is maintained by collagen including a blood vessel, and the embrittlement of an organ it not only only advances aging of the skin, but, and becomes a cause which causes trouble healthily. However, collagen is protein, and only by applying to an ingestion or a skin surface, since the inside of the body is hard to be absorbed directly, furthermore the activity of fibroblast or keratinocyte cannot be enhanced, it cannot become fundamental prevention and therapy of aging of the skin. Then, it is possible to maintain and reinforce production of collagen which is the main constituents of dermis, or an organization and an organ for the purpose of the aging prevention of the skin, maintenance, improvement of health, etc., And development of a safe collagen production enhancement agent and the activator of the keratinocyte itself which exists in the fibroblast which exists in the dermis which constitutes the skin further, or epidermis is desired.

[0007]

[Problem(s) to be Solved by the Invention]In view of this situation, there is a technical problem of this invention in providing a means to reinforce the collagen production by L-ASUKORUBIN effectively.

[0008]

[Means for Solving the Problem]As a result of repeating examination and search for solving the above-mentioned technical problem using fibroblast, this invention persons by combining L-ascorbic acid and royal jelly, In concentration the production is not accepted to be by use of L-ascorbic acid themselves in collagen production by L-ascorbic acid, or concentration only a low production amount is accepted to be, unexpected knowledge that the production reinforced

efficiently was reached by adding royal jelly. If inactivation of L-ascorbic acid progresses and it is already independent as the result, even if it is a case where collagen production is not accepted, By making royal jelly exist, the activity of L-ascorbic acid was enhanced and it found out as original knowledge that collagen production manifested itself. Transforming by which collagen production of fibroblast is secreted from fibroblast etc. on the other hand Growth Being reinforced by factor (it is hereafter written as "TGF-beta".) was known. As a result of this invention persons' advancing research further also paying attention to this point, royal jelly to fibroblast under existence of ascorbic acid, To keratinocyte of epidermis which reinforces production of TGF-beta, in addition exists in the surface side of the skin rather than dermis, it is low SUYARU jelly themselves and found out reinforcing production of TGF-beta. Thus, it checks that one of the collagen production enhancement mechanisms of the L-ascorbic acid by royal jelly is based on enhancement of production of TGF-beta, Royal jelly and/or royal jelly, and L-ascorbic acid found out that it was useful as a collagen production enhancement agent, a TGF-beta production enhancement agent, and/or a keratinocyte growth accelerator, and they completed this invention.

[0009]Namely, this invention is what solves the above-mentioned technical problem by providing a collagen production enhancement agent containing L-ascorbic acid and royal jelly, its manufacturing method, and a use, An aforementioned problem is solved by providing a constituent containing a collagen production enhancement agent containing L-ascorbic acid and royal jelly, its manufacturing method, and a use.

[0010]This invention solves an aforementioned problem by providing a TGF-beta production enhancement agent containing royal jelly or royal jelly, and ascorbic acid and a keratinocyte growth accelerator, and its manufacturing method row use.

[0011]

[Embodiment of the Invention]The collagen production enhancement agent of this invention contains L-ascorbic acid and royal jelly. With the L-ascorbic acid as used in the field of this invention, L-ascorbic acid and/or its salt, L-ascorbic acid 2-glycosides including L-ascorbic acid 2-glucoside, They are L-ascorbic acid derivatives and/or those salts, such as L-ascorbic acid phosphoric acid, L-ascorbic acid sulfuric acid, DL-alpha-tocopherol 2-L-ascorbic acid diester phosphate, an acylation derivative of L-ascorbic acid 2-glucoside, Any may be sufficient, as long as what is necessary is to demonstrate the physiology activity of L-ascorbic acid in the living body, and just to have combined one sort of those compounds, or two sorts or more and it is a thing of the gestalt of a constituent and a mixture. The salts of the L-ascorbic acid mentioned as L-ascorbic acid as used in the field of this invention may be one sort of inorganic bases, such as sodium, potassium, calcium, magnesium, ammonium, and alkylammonium, and/or an organic base, or two sorts or more of which combination. There is ascorbic acid and stable type L-ascorbic acid, such as L-ascorbic acid 2-glycoside, Even if it medicates the skin,

do not receive oxidative degradation and, moreover, it is gradually decomposed by a living body's enzyme, Since it had the sustained-release effect, when the frequency of administration to a living body can be reduced and a medicine is further prescribed for the patient, since the effect as L-ascorbic acid continues for a long period of time as compared with L-ascorbic acid, it is desirable especially as L-ascorbic acid of the collagen production enhancement agent of this invention.

[0012]Although this specification did not show the concrete experimental result, when an animal including people took in said each of ascorbic acid in taking orally, or when a medicine was endermically prescribed for the patient, all were absorbed by in the living body as ascorbic acid, and it was checked that a collagen production operation is shown. If it is in the feed for animals which can compound L-ascorbic acid in a body, a feed, pet food, etc., L-gulono gamma-lactone which is a precursor of L-ascorbic acid changed into L-ascorbic acid in the living body can also be used as ascorbic acid of this invention.

[0013]The opalescence of royal jelly given as food to the larva of a queen bee which was secreted by the worker of the honeybee and accumulated in the royal cell of the nest is liquefied. With and the royal jelly as used in the field of this invention. As long as it is a constituent manufactured considering royal jelly or royal jelly as a raw material and reinforces and deals in the collagen production by L-ascorbic acid, even if nature is still liquefied, it, It may be which thing of the gestalt of the solid except [liquefied or liquefied] having added processing artificially, powder, granulation, a paste, etc. There is no limitation in particular in the kind of honeybee which royal jelly secretes, or its origin. As a kind of the honeybee to secrete, an Apis mellifrera (Apismellifera), a TOUYOU honeybee (Apiscerana), OOMITSUBACHI (Apisdorsata), handicap TSUBACHI (Apisflorea), etc. are mentioned. As an origin, Japan, South America, North America, Australia, China, Europe, etc. are mentioned. Generally, royal jelly may start adverse effects, such as an allergic reaction, depending on the individual made into the object of use of it. Since South America, division, and the royal jelly from Brazil have comparatively few such adverse effects, they are useful to especially operation of this invention. As for processing of not less than 65 **, since denaturation arises for the ingredient with heating, avoiding is common [raw royal jelly (henceforth "non-heat-treating royal jelly")], as shown in JP,60-9457,A. However, since the operation which reinforces the collagen production by L-ascorbic acid is not deactivated, the royal jelly (henceforth "heat-treatment royal jelly") heated for more than 30 minutes above 70 ** as a material used by this invention, Germicidal treatment can be carried out by heat-treatment, or for example, it is not desirable for a living body, the heat-treatment royal jelly which denatured the protein ingredient etc. which have allergenic nature, and decreases thru/or vanished allergenic nature can also be used advantageously. As royal jelly of this invention, By using the method of solvent extraction, such as non-heat-treating royal jelly or not only heat-treatment

royal jelly but acetone, ethanol, water, etc., gel filtration, and others, It is the preparation which refined the royal jelly concerned selectively, and they can also be used if it has the operation which reinforces the collagen production by L-ascorbic acid. Hereafter, especially in this specification, unless it refuses, non-heat-treating royal jelly and heat-treatment royal jelly are only collectively called "royal jelly."

[0014]The collagen production enhancement agent of this invention contains the above L-ascorbic acid and royal jelly. The loadings with the L-ascorbic acid and royal jelly in the collagen production enhancement agent of this invention should just contain the L-ascorbic acid and royal jelly more than the required minimum quantity with which collagen production of L-ascorbic acid is reinforced by royal jelly. The operation which reinforces collagen production of L-ascorbic acid by royal jelly can be judged by the method of measuring the collagen production amount in the fibroblast of the hamster newborn infant who shows the experiment mentioned later, for example. Incidentally the collagen production enhancement agent of this invention L-ascorbic acid 0.02% of the weight or more by the weight conversion as L-ascorbic acid per the gross weight desirably, It contains 0.05% of the weight or more, and 0.5 or more weight sections of royal jelly [one or more weight sections of] is desirably blended by the weight conversion to the L-ascorbic acid 1 weight section concerned.

[0015]The collagen production enhancement agent of this invention does not bar adding an anti-oxidant. As for the anti-oxidant in this case, what controls the oxidative degradation under preservation of the ascorbic acid in the collagen production enhancement agent of this invention is desirable, and, thereby, stabilization with the higher level of the collagen production enhancement agent concerned can be attained. When using the collagen production enhancement agent of this invention as edible [for animals including Homo sapiens], it can choose from what is usually used by a food field suitably as an anti-oxidant. Specifically, flavonoid, polyphenol, vitamin E, etc. can use advantageously as an anti-oxidant in this invention. Although the content in particular in the collagen production enhancement agent concerned of these anti-oxidants does not have restriction, When using the collagen production enhancement agent concerned as edible in consideration of the influence on taste, it is desirable to reduce a little and for these anti-oxidants to use from it according to the blending ratio usually used, by a food field. When using the collagen production enhancement agent of this invention in a cosmetic field, the quasi-drugs field, or the drugs field, it is desirable to reduce a little and to use from it the anti-oxidant usually used respectively according to the blending ratio by which normal use is carried out, in the field concerned.

[0016]To the collagen production enhancement agent of this invention, grape sugar, fructose, lactose, Sugars, such as trehalose, malt sugar, sucrose, RAKUTO sucrose, and a starch syrup, Annular sugars, such as annular tetrasaccharide indicated by the international publication WO 02/No. 10361 gazette (name of an invention "alpha **ISO malto sill GURUKO

sugar generation enzyme, its manufacturing method, and use") by the cyclodextrin or the same applicant, Erythritol, mannitol, sorbitol, xylitol, maltitol, Sugar-alcohol, such as a restoration water candy, Aspartame, stevia extract, By adding one sort, such as thickeners, such as carboxymethyl cellulose of polysaccharide, such as sweeteners of high sweetness, such as a SHUKURA sirloin and Acesulfam K, pullulan, and a carrageenan, natural gums, and synthetic compounds, or two sorts or more, If it is in a solid thing, it not only can use in favor of the formativeness, but it can use in favor of stabilization of the collagen production enhancement agent of this invention, a taste improvement, flavor maintenance, etc.

[0017]An ingredient with collagen production potentiation, such as an extract from things other than royal jelly and L-ascorbic acid, for example, the leaf bud of the Fagaceae Buna group currently indicated by JP,10-203952,A, can also be blended with the collagen production enhancement agent of this invention if needed. Furthermore, if needed An emulsifier, perfume, spices, and coloring matter, for example, vitamin B_1, One sort or also making two or more sorts contain can carry out advantageously the ingredient except the above of vitamins other than L-ascorbic acid, such as vitamin B_2, vitamin B_6, vitamin E, vitamin P, or those derivatives, and amino acid having described. The selection criterion of these ingredients is usually suitably chosen according to the necessity for each field of the invention of the collagen production enhancement agent of this invention. There is no restriction in particular in the gestalt of the collagen production enhancement agent of this invention containing the above ingredients, and it is provided with the gestalt of a request of powder, granulation, a tablet, a paste, jelly, a milky lotion, a solution, etc.

[0018]the collagen production enhancement agent of this invention in it to animals including Homo sapiens. [taking-orally] Since the active principle maintains and reinforces the collagen production by the L-ascorbic acid in the fibroblast which is promptly absorbed by in the living body and exists in dermis, an organization, an organ, etc. also when a medicine is prescribed for the patient in which an endermic course, If animals including Homo sapiens take in the collagen production enhancement agent concerned, production of a cola gene will continue stably, The aging accompanying aging and the fall of the collagen production ability of the skin which received the damage by factors including ultraviolet rays can be recovered, a beam and grace can be given to the skin, small JIWA wrinkles can be removed, and the effect of recovering the elasticity of the skin can be done so. If it is in dermal administration especially, L-ascorbic acid and royal jelly reach promptly the keratinocyte which exists in the fibroblast which exists in dermis, or epidermis, and they reinforce production of TGF-beta, While maintaining and reinforcing the collagen production by L-ascorbic acid, Royal jelly promotes growth of keratinocyte and it the cornified layer, Since it strengthens and biophylaxis ability is reinforced, the fall of the collagen production ability of the skin which received the damage by factors including aging, ultraviolet rays, injurious microorganisms, etc. accompanying aging is

recovered promptly, It is very effective for giving a beam and grace to the skin, removing small JIWA wrinkles, and recovering the elasticity of the skin. And an animal including Homo sapiens can use the collagen production enhancement agent of this invention simple, It can use also for maintenance and improvement of health, for example, divides also as a tonic, a TGF-beta production enhancement agent, a keratinocyte growth accelerator, health food, a health supplement, a food for specified dietary use, a food with health claims, cosmetics, quasi drugs, drugs, feed, a feed, pet food, general merchandise, etc., and is useful.

[0019]The intake or the dose of a collagen production enhancement agent of this invention, Although it changes with the kind of animals including target Homo sapiens, pet, etc., age, sex, etc., by the weight conversion as L-ascorbic acid. per weight of 1 kg -- usually -- 0.1 mg -- or desirably 0.25 g, 1 mg thru/or 0.5 g, and the weight conversion as royal jelly -- per weight of 1 kg -- usually -- 0.5 mg thru/or 2 g -- desirable -- 1 mg -- or what is necessary is to set and take in every day or the interval of the 1st day or more in 1 time per day, or several steps according to an effect, or just to prescribe 1 g for the patient in taking orally In the tonic, the health food, the health supplement, the food for specified dietary use, the food with health claims and quasi drugs which are prescribed for the patient in taking orally, drugs, feed, a feed, and pet food. For example, liquids and solutions, a tablet, powders, a granule, a paste agent, a syrup agent, a capsule, etc. can use the thing of a gestalt according to each use. In applying the collagen production enhancement agent of this invention to the skin directly as skin external preparations, such as cosmetics, The L-ascorbic acid or royal jelly used for the collagen production enhancement agent concerned, By the weight conversion as L-ascorbic acid or royal jelly, 0.001 thru/or 20% of the weight among the skin external-preparations whole quantity respectively preferably, What is necessary is to be 0.005 % of the weight thru/or 15 % of the weight, to set every day or the interval of the 1st day or more in 1 time per or several steps according to an effect day, and just to apply to the skin directly. At less than 0.001 % of the weight, if the effect is in the product which becomes that it is hard to be demonstrated and exceeds 30 % of the weight, it may not be preferred in respect of the physical properties of a product. [of L-ascorbic acid or royal jelly] A lotion, a milky lotion, cream, solid, powder, jelly, a pack, a face mask, etc. can be used for the skin external preparations concerned as a thing of the gestalt according to the purpose of use, for example.

[0020]The collagen production enhancement agent of this invention can be advantageously used also as a gestalt of the constituent blended with other ingredients, while it is useful at itself, as stated above. In order to manufacture the constituent containing the collagen production enhancement agent of this invention, According to the proper presentation chosen according to the target animals, the ingestion method or a medication method of those, etc., ascorbic acid and royal jelly. An eating-and-drinking article, cosmetics, quasi drugs, drugs, feed, a feed as shown above, One sort or two sorts or more of ingredients used for any of the

field of pet food they are setting, What is necessary is to mix according to the purpose, to carry out suitably processes, such as dilution, concentration, desiccation, filtration, and centrifugal separation, based on each content, to prepare the constituent containing L-ascorbic acid and royal jelly, and just to fabricate in desired shape if needed. An order which blends each ingredient, and the stage to carry out the process concerned, What is necessary is for there to be no restriction, for example, to be fresh as much as possible, or to mix L-ascorbic acid in the non-heat-treating royal jelly by which after-extraction cold storage was carried out, and just to carry out the process concerned suitably after that if needed, unless the quality degradation of L-ascorbic acid and royal jelly is caused. As for combination of L-ascorbic acid, since ascorbic acid is unstable with heat, when using heat-treatment royal jelly, it is preferred to carry out, after heat-treating non-heat-treating royal jelly. As an ingredient to the animal which is used by this invention and including Homo sapiens of which endermic application or skin external use is permitted [taking orally], By each field of the invention of the constituent of this invention, normal use is carried out, for example, water, alcohol, starch, protein, amino acid, a fiber, sugar, lipid, fatty acid, a vitamin, a mineral, flavors, a coloring agent, sweetners, a seasoning, spices, an antiseptic, an emulsifier, a surface-active agent, etc. are mentioned. The constituent by this invention can be advantageously used with a food field, the drink field (the feed of an animal, a feed, and pet food are included), foods for specified dietary use, foods with health claims, a cosmetic field, the quasi-drugs field, the drugs field, general merchandise, etc., for example.

[0021]After the collagen production enhancement agent of this invention of a solid gestalt mixes L-ascorbic acid and non-heat-treating royal jelly and mixes other ingredients further if needed, for example, it can obtain the mixture concerned by presenting the usual drying processes, such as reduced pressure drying, vacuum drying, and warm air desiccation. The collagen production enhancement agent concerned of a solid gestalt can also be obtained by using as an excipient anhydrous [which is indicated by JP,6-170221,A by the same applicant, for example / alpha], alpha-trehalose, etc., without passing through the usual drying process. Namely, what is necessary is to add a crystal or amorphous alpha, and alpha-trehalose anhydride into the mixture of L-ascorbic acid and non-heat-treating royal jelly, and just to settle the mixture concerned below at ordinary temperature. What was prepared without passing through the usual drying process using the anhydride of alpha and alpha-trehalose, Since the stability of various operations which non-heat-treating royal jelly has not to mention the operation which reinforces collagen production of the L-ascorbic acid by non-heat-treating royal jelly divides and is excellent, it can use in favor of this invention. Using the gestalt of requests, such as powder, granulation, and a tablet, or filling up a capsule with this powder or this granulation, and using it further, using a grinder, a granulator, a tableting machine, etc., if needed, can also carry out advantageously the collagen production enhancement agent

concerned of the gestalt of these solid states. The dehydrator used for the disintegration of the collagen production enhancement agent of this invention, Holding the activity of royal jelly stably, if it is an edible dehydrator which can be dried, any may be sufficient and the annular tetrasaccharide of anhydrous [alpha], beta-trehalose, anhydrous malt sugar, anhydrous, or 1 water, etc. will be preferably illustrated besides anhydrous [alpha] and alpha-trehalose.
[0022]There is no restriction in particular in the gestalt of the constituent of this invention. As desirable foodstuffs, for example Ice cream, a Popsicle, Syrup, such as ice cream, such as sherbet, and ice syrup, butter cream, custard cream, Spreads, such as a flower paste, a peanut paste, and a fruit paste, and a paste, Chocolate, jelly, a candy, oleaster jelly, a caramel, chewing gum, Western-style cakes, such as a pudding, a cream puff, and a sponge cake, jam, marmalade, Processed fruits, such as syrup ** and a confection, or processing vegetables, steamed filled dumplings, sweet rice paste, Seasonings, such as Japanese sweets, such as bean jam, sweet bean paste, sweet jellied bean paste, sponge cake, a candy ball, and a rice confectionery, soy sauce, powdered soy sauce, bean paste, powdered miso, mayonnaise, a dressing, vinegar, a mixture of vinegar, soy sauce, sake, mirin or sugar, table sugar, and coffee sugar, etc. are mentioned. As a gestalt of a desirable drink, soft drinks, such as tea drinks, such as alcoholic beverages, such as synthetic sake, brewage, fruit wine, and wine, juice, a mineral drink, a carbonated drink, a lactic acid drink, a lactic acid bacteria beverage, an isotonic drink, drinkable preparations, green tea, tea, oolong tea, coffee, and cocoa, etc. are mentioned, for example. As a gestalt of desirable cosmetics, for example A lotion, a milky lotion, cream, The basic cosmetics of the gestalt of solid, powder, jelly, a pack, a face mask, etc., The cosmetics for washing, the cosmetics for bathing, mouth cosmetics, suntan and sunscreen cosmetics, makeup cosmetics, hair cosmetics, and the general merchandise that have direct influence on skin like detergent for kitchen (a hair-growth agent, a hair restorer, etc.) are mentioned. What is necessary is to add the collagen production enhancement agent of this invention at the proper stage of the process in which the product made into the purpose is manufactured in accordance with a conventional manufacturing method, or just to add L-ascorbic acid and royal jelly suitably separately, in order to manufacture the constituent by above this inventions. Although there is no restriction in particular at the stage of addition, Although the target product is manufactured through a heating process, to a case, attenuation of the collagen production potentiation in a manufacturing process can be prevented after a heating process about an ingredient unstable with L-ascorbic acid and other heat ordinary temperature and by adding desirably, after cooling at 30 ** or less. the constituent of above this inventions -- the collagen production enhancement agent of this invention -- per product weight -- usually -- 0.01 % of the weight thru/or 20 % of the weight -- desirable -- 0.1 % of the weight -- or it contains 10% of the weight.

[0023]In this invention, when L-ascorbic acid is L-ascorbic acid derivatives, such as L-ascorbic acid 2-glycoside, It is cut by operation of the enzyme etc. which exist in in the living body, cell surface, etc., In being salts of L-ascorbic acid, such as L-ascorbic acid potassium, it dissociates to ion, and each L-ascorbic acid taken in or prescribed for the patient reaches the keratinocyte of the fibroblast and the epidermis which exist in dermis, an organ, etc. as L-ascorbic acid. And the royal jelly absorbed by in the living body simultaneously with L-ascorbic acid acts on fibroblast and/or keratinocyte, and it reinforces production of TGF-beta, By showing the operation which reinforces the collagen production by L-ascorbic acid of fibroblast, if possible, it closes that the collagen production potentiation concerned continues. Therefore, by using the collagen production enhancement agent concerned daily like the usual product, Since the potentiation of collagen production is effectively demonstrated in the living body which used and the collagen production by L-ascorbic acid is maintained for a long period of time, if animals including Homo sapiens take in, production of collagen, such as fibroblast distributed over dermis, other organizations, or an organ, will continue stably. Royal jelly acts on keratinocyte, it reinforces production of the TGF-beta, and it promotes growth of keratinocyte, strengthens the skin and reinforces the biophylaxis ability. As a result, recover the fall of the collagen production ability of the fibroblast of dermis to the skin which received the damage by the aging accompanying aging, and factors including ultraviolet rays, and. The biophylaxis ability of epidermis can be strengthened, a beam and grace can be given to the skin, small JIWA, the improvement of wrinkles, prevention of generating, and elasticity can be recovered, and the effect which strengthens the organization of internal organs or a blood vessel can be done so. Since the constituent concerned contains L-ascorbic acid and royal jelly, It not only has an effect which reinforces production of collagen by L-ascorbic acid, but, It cannot be overemphasized that L-ascorbic acid and the royal jelly of each also combine the improvement of the surface deterioration of enhancement of the resistance force of the whole living body which originally has, the improvement of early-stage-izing of an improvement of poor health, the healthy maintenance effect of a state, and surface deterioration and the various skin disease accompanying it, and a healthy person, roughness nature, etc., and they are attained. Therefore, the foodstuffs and the drink for it being not only useful in order to maintain the healthy skin where the constituent of this invention prevents aging of the skin and which does not have wrinkles, but maintaining and increasing cosmetics and health, It is very useful as a food for specified dietary use, a food with health claims, cosmetics, quasi drugs, drugs, feed, a feed, pet food, general merchandise, etc. And also when using as cosmetics applied to the skin containing the scalp, it takes effect in the improvement of a curative effect to prevention and the disease concerned of the skin disease by the above-mentioned operation effect, growth hair, etc. When applying to the skin directly by using the constituent containing the collagen production enhancement agent of this invention, and/or it as cosmetics etc.,

Osmosis on the skin may be promoted by using the iontophoresis implement indicated by the international publication WO 01/No. 60388 gazette (application for patent No. 80195 [2001 to]) (the name of an invention, an "iontophoresis implement") by the same applicant, etc. if needed for example. The operation which reinforces collagen production of the L-ascorbic acid by the royal jelly of this invention, Since it has the stable feature held even if it processes non-heat-treating royal jelly for more than 30 minutes above 70 **, in the point, it is necessary to pay attention in particular for neither preparation of the constituent which made the collagen production enhancement agent of this invention contain, nor the stability at the time of preservation. However, non-heat-treating royal jelly, Since there is a problem that quality at large [the] originally deteriorates easily in the state as it is where it was extracted, and the most useful operation declines promptly, For example, as indicated by the application-for-patent No. 37200 [2000 to] specification (the name of an invention, a "cell activator") by the same applicant, It can also use advantageously ambient air temperature being able to control degradation of the quality in the retention period in about 25 **, and handling using the constituent of an easy gestalt, and preparing the collagen production enhancement agent of this invention by adding alpha and alpha-trehalose.

[0024]Hereafter, based on an experiment and an example, this invention is explained more to details.

[0025]

[Experiment 1] As <examination of the collagen production operation by L-ascorbic acid or royal jelly> ** ascorbic acid ascorbic acid, sodium L-ascorbate (special grade chemical Wako, Inc. sale) was used.

** In the royal jelly book experiment, non-heat-treating royal jelly and heat-treatment royal jelly were used as royal jelly. As non-heat-treating royal jelly, the raw royal jelly from Brazil (it saves with the moisture of 67 % of the weight-20 **.) was thawed at ordinary temperature at any time on the occasion of use, and the initial complement was subdivided and used immediately after. Heat-treatment royal jelly said non-heat-treating royal jelly, It extracted 5g at a time in the glass test tube whose caliber is 18 mm, in 40 **, 50 **, 60 **, 70 **, 80 **, and 90 ** processing, held for 30 minutes in the thermostat, and, in 100 ** processing, held for 30 minutes in the oil bath. The experiment after cooling at 30 ** or less was promptly presented after heat-treatment.

** In accordance with the preparation conventional methods of hamster newborn infant fibroblast, the hamster newborn infant's regions-of-back skin was cut open, and fibroblast was isolated from the exfoliative section of the skin. Hereafter, the method is outlined. Epidermis and dermis were exfoliated after settlement at 4 ** in the section concerned overnight in the culture medium which dissolved dispase (Godo Shusei Sale) in the MEM culture medium (Nissui Sale) of the eagle containing 0.03mM Ca_2^{++} beforehand so that it might be set to ml in 500 units /. It held at 37 ** for 1 hour in Dulbecco's MEM culture medium (it outlines Nissui

Sale and the following "D-MEM".) which dissolved collagenase (Amano Pharmaceuticals Sale) so that it might become 0.25% (v/v) about desquamating dermis. Then, the suspension of single cell of hamster newborn infant fibroblast preparation-back was collected, and centrifugal separation recovered the cell by carrying out pipetting of the split of dermis in the culture medium. The collected cell is suspended to phosphate-buffered saline, and after 30-minute neglect, centrifuge the supernatant liquid containing fibroblast and the cells concerned are collected, It was re-suspended to 10% (v/v) of fetal-calf-serum (it outlines Gibco BRL sale and the following "FCS".) content D-MEM, and was used as fibroblast for future collagen production amount measurement.

** Culture of the cell below measurement of the collagen production by L-ascorbic acid was performed in the incubator of 37 ** and 5%(v/v) CO_2 concentration. On the plate of six wells, 3 ml of the hamster newborn infant's fibroblast suspension prepared by the aforementioned ** was planted so that it might become a $4x10^5$ cell / well, and on it, it was cultivated for seven days. To D-MEM containing 10% (v/v) of FCS, sodium L-ascorbate by the weight conversion as L-ascorbic acid. It dissolved so that it might become respectively in 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 and 50.0, and ml and 100.0 or 200.0 microg /. Similarly 10% (v/v) of FCS to contained D-MEM non-heat-treating royal jelly or heat-treatment royal jelly, It dissolved so that it might become respectively in 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0, and ml and 200.0 or 500.0 microg /. In addition to the well which removed the culture supernatant of the fibroblast concerned, 5 ml of each culture media which dissolved the sodium L-ascorbate concerned or royal jelly were further cultivated for three days. The culture supernatant of each well Then, the same ascorbic acid, or the ascorbic acid same after replacing by 5 ml of culture media prepared to royal jelly concentration and cultivating for three days as the above -- or, It replaced by 1 ml of culture media of royal jelly concentration, and after 3 more hour culture, it added so that it might become 3 microcurie / well, and the [2,3-^{3}H] proline (40 Ci/mmol, Amersham sale) which dissolved in D-MEM was cultivated further overnight. Each culture medium (L-ascorbic acid or the thing of royal jelly addition is included) used for this experiment used what was filtered with a 0.22-micrometer filter.

** By the fixed-quantity aforementioned ** of the proline incorporated into the collagen which fibroblast produced under existence of [2,3-^{3}H] proline, After culturing a hamster newborn infant's fibroblast overnight, under [a fixed quantity / proline / which extracted collagen and was incorporated from the cell in accordance with the conventional method / [2,3-^{3}H]]. A fixed quantity of proline is Theo Jin. It carried out based on Kim et al. (Seong-Jin Kim), "dermatologic Sir jelly" (Dermatologic Surgery), the 24th volume, and the method indicated to the 1054 - 1058th page (1998). the removing-hereafter,-supernatant liquid of cell which added [2,3-^{3}H] proline and was cultured by aforementioned ** overnight back if the method is outlined

-- each -- to a well. 0.3 ml of trypsin (Gibco BRL sale) / well was added, and it settled for 10 minutes at 37 **, and further, 0.3-ml D-MEM was added and the cell was suspended to the culture medium concerned. The cell suspension concerned was centrifuged, supernatant liquid was removed, and fibroblasts were collected, and 0.1 ml of 1mg/ml of pepsin (sigma company sale) content 1M acetic acid was added into the cell concerned, and it agitated and mixed into it, and shook at the room temperature for 4 hours. Next, 0.8 ml of 200 microg/ml type I Laa Genn (Koken Co., Ltd. sale) content 0.5M acetic acid was added, 5M sodium chloride adjusted supernatant liquid to 0.15M after centrifugal separation for 5 minutes at 3,000 revolutions per minute and 4 **, and it centrifuged for 10 minutes at 12,000 revolutions per minute and 4 **. Supernatant liquid was adjusted to 0.45M with 5M sodium chloride, and centrifugal separation Kiyoshi Gokami was removed for 30 minutes at 3,200 revolutions per minute and 4 **. 4 ml of ethanol solutions were added 20% (v/v) to precipitation, and centrifugal separation Kiyoshi Gokami was removed for 10 minutes at 3,200 revolutions per minute and 4 ** to it. Finally, 0.25 ml added to precipitation, 0.5M acetic acid was agitated to it, precipitation was suspended, the suspension was suspended in a 5-ml scintillation solution, and the quantity of the ^{3}H proline incorporated into collagen was quantified in accordance with the conventional method with the liquid scintillation counter. the experiment experimented about sodium L-ascorbate and the each concentration of royal jelly using three wells.

[0026]A collagen production amount (counted value of a liquid scintillation counter) when not adding L-ascorbic acid for the relation of the addition of L-ascorbic acid and the collagen production amount according the result to a hamster newborn infant's fibroblast is shown in Table 1 with the relative value set to 100. A hamster newborn infant's fibroblast sodium L-ascorbate in an additive-free case. When L-ascorbic acid was added to a low level having collagen production, the increase in the collagen production amount depending on the concentration was accepted, and the collagen production by L-ascorbic acid was checked. The minimum effective dose of L-ascorbic acid for the collagen production in this experiment system was 0.5 microg/ml, in 50 microg [more than]/ml concentration, the collagen production amount reached the saturation point and the significant increase was not observed in a collagen production amount in the concentration beyond it. Even if it was the concentration of the gap to use for this experiment if royal jelly was independent irrespective of the existence of un-heat-treating and heat-treatment although concrete data was not shown, enhancement of collagen production was not accepted at all.

[0027]

[Table 1]

L－アスコルビン酸濃度（μg／ml）	相対的なコラーゲン産生量 *
0．0	100±12
0．1	95±13
0．2	118±26
0．5	210±33 **
1．0	482±89 **
2．0	1497±155 **
5．0	1755±151 **
10．0	2089±160 **
20．0	2456±186 **
50．0	2841±341 **
100．0	2956±500 **
200．0	2863±456 **

*：相対値±標準偏差

**：有意差有り（P＜0．05）

[0028]

[Experiment 2] Royal jelly investigated the influence which it has on the collagen production by L-ascorbic acid using the system of measurement of the collagen production amount used for the <examination [of the influence which it has on the collagen production by the L-ascorbic acid of royal jelly] **> experiment 1. The fibroblast of the newborn infant hamster prepared by the same method as the experiment 1 was cultured between seven days of plant lumps on the plate of six wells. Sodium L-ascorbate to D-MEM which contained 10% (v/v) of FCS after removing the culture supernatant of the well concerned by the weight conversion as L-ascorbic acid. It dissolves so that the last concentration may become respectively in 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0, and ml and 50.0 or 100.0 microg /, The culture medium [each] which dissolved non-heat-treating royal jelly or heat-treatment royal jelly of the solution so that it might become respectively in 0.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0, and ml and 200.0 or 500.0 microg /is added by 5 ml / well, The collagen production amount was measured by the same method as the experiment 1. The experiment used three wells about each concentration. It was authorized whether a result would consider counted value of the royal jelly additive-free liquid scintillation counter in each L-ascorbic acid concentration as contrast, and the counted value of contrast and each royal jelly addition concentration would have a significant difference. In the test of significance, 0.1> P>"+" 0.05 were expressed as "**", P> 0.1 was expressed for P< 0.05 as "-", it had "+", and it was judged that royal jelly reinforced the collagen production by L-ascorbic acid. In the density range of the royal jelly which the collagen production amount gave to saturation more than in 50 microg/ml, and the

concentration of L-ascorbic acid used for the experiment this time, the enhancement beyond it of a collagen production amount was not accepted. Non-heat-treating royal jelly and heat-treatment royal jelly, Since the difference was not observed in the operation which reinforces the collagen production by L-ascorbic acid with any [40 **, 50 **, 60 **, 70 **, 80 **, 90 **, and 100 **] treatment temperature, A result is a case of non-heat-treating royal jelly, and showed only the result whose L-ascorbic acid concentration is to 0 thru/or 20 microg/ml as Table 2.

[0029]

[Table 2]

	L-アスコルビン酸濃度（μg/ml）								
	0.0	0.1	0.2	0.5	1.0	2.0	5.0	10.0	20.0
0.0	−	−	−	−	−	−	−	−	−
2.0	−	−	−	−	−	−	−	−	−
5.0	−	−	−	−	−	−	−	±	±
10.0	−	−	−	±	+	+	+	+	+
20.0	−	−	±	+	+	+	+	+	+
50.0	−	−	+	+	+	+	+	+	+
100.0	−	−	+	+	+	+	+	+	+
200.0	−	−	+	+	+	+	+	+	+
500.0	−	−	+	+	+	+	+	+	+

[0030]it was shown in the experiment 1 -- as -- L-ascorbic acid -- if independent and the concentration is not 0.5 micro more thang/ml, in spite of not accepting a collagen production operation, by using royal jelly together, it will be 0.2 microg/ml concentration, and a collagen production operation will already be accepted. Since L-ascorbic acid is not contained in the royal jelly used in the experiment, royal jelly from this result, Also having the operation which is reinforcing the collagen production ability by L-ASUKORUBI acid, and makes concentration of L-ascorbic acid required for a collagen production operation lower than an L-ascorbic acid independent case by ingredients other than L-ascorbic acid was checked. In addition, at least, when L-ascorbic acid was the concentration which is 20.0 microg/ml, when adding 10 microg [more than]/ml royal jelly, it was checked that the collagen production by L-ascorbic acid is reinforced with royal jelly. This receives decomposition in the living body, and if ascorbic acid is use independently, Even when it becomes the concentration which cannot demonstrate the collagen production operation thru/or to which production ability falls, the collagen production by ascorbic acid is reinforced and by using royal jelly together shows that the production ability of the collagen concerned can maintain stably.

[0031]

[Experiment 3] <Examination [of the influence which it has on the collagen production by the L-ascorbic acid of royal jelly] **> experiment 2 under existence of the 200 microg/ml royal jelly in which it set and enhancement of the collagen production by L-ascorbic acid was accepted, The influence which it has on collagen production using sodium L-ascorbate and L-ascorbic

acid 2-glucoside was considered.
** As L-ascorbic acid L-ascorbic acid, sodium L-ascorbate (the same thing as the experiment 1) and L-ascorbic acid 2-glucoside (the Hayashibara Co., Ltd. biochemistry research institute sale) were used.
** The royal jelly of same not heating it as having used it in the experiment 1 was used for royal jelly royal jelly.
** To the D-MEM culture medium containing FCS of 10% (v/v) of measurement of the collagen production by sodium L-ascorbate or L-ascorbic acid 2-glucoside. Royal jelly was dissolved so that it might become in ml and 200 microg /, and further, L-ascorbic acid 2-glucoside was dissolved in the solution so that it might become respectively by the weight conversion as L-ascorbic acid in 0.2, 0.5, and ml and 1.0 microg /. As contrast, by the weight conversion as L-ascorbic acid, L-ascorbic acid 2-glucoside was dissolved in the D-MEM culture medium containing 10% (v/v) of FCS, and the royal jelly additive-free culture medium so that it might become respectively in 0.2, 0.5, and ml and 1.0 microg /. the culture medium which dissolved the L-ascorbic acid 2-glucoside concerned and royal jelly -- or, Under [a fixed quantity / method / as the experiment 1 / same / production amount / of collagen / in addition to the well which removed the culture supernatant, it cultures hamster newborn infant fibroblast for 5 ml of each culture media prepared as contrast for seven more days after culture between seven on the plate of six wells like the experiment 1 and]. As negative contrast, with 200 microg/ml royal jelly, the culture medium which dissolved sodium L-ascorbate by the weight conversion as L-ascorbic acid so that it might become in ml and 1 microg /was added similarly, and culture was performed for seven days.
[0032]The result The addition of L-ascorbic acid 2-glucoside under royal jelly existence and nonexistence, With the relative value set to 100, the collagen production amount of fibroblast in case neither L-ascorbic acid nor royal jelly ** the relation of the collagen production by hamster newborn infant fibroblast is shown in Table 3. Hamster newborn infant fibroblast was a low level like [in an additive-free case / production of collagen] the object of the experiment 1 about L-ascorbic acid 2-glucoside irrespective of the existence of addition of royal jelly. When the ascorbic acid 2-glucoside of 0.2 microg [more than]/ml concentration was added, collagen production was reinforced as compared with additive-free, and the production amount of collagen was further reinforced by addition of royal jelly. Although concrete data is not shown, on the other hand, in the experiment 1. When 1 microg/ml L-ascorbic acid is added, it cultivates for three days and it cultivates for three more days after substitution by the same culture medium, Since it had not replaced by the same culture medium after cultivating for three days in spite of having cultivated 1 microg/ml L-ascorbic acid by the added culture medium to collagen production having been accepted at the negative control, enhancement of collagen production was not accepted. The L-ascorbic acid 2-glucoside which dissolved in the

culture medium this in a culture medium, Since it is gradually decomposed into L-ascorbic acid and glucose by the glucosidase which is very stable and exists in a cell as compared with L-ascorbic acid, Since the activity as L-ascorbic acid continued to stability for seven days, it thinks, and the predominance of L-ascorbic acid 2-glucoside at the time of combining royal jelly and ascorbic acid is shown.

[0033]

[Table 3]

L－アスコルビン酸２－グルコシド濃度（μｇ／ｍｌ）	ローヤルゼリー添加の有無（２００μｇ／ｍｌ）	コラーゲン産生量 *
0.0	無	100±23
	有	134±34
0.2	無	219±31
	有	298±30 **
0.5	無	527±17
	有	1440±70 **
1.0	無	2250±436
	有	3168±318 **

*：相対値±標準偏差

**：同濃度のＬ－アスコルビン酸２－グルコシド添加区において、ローヤルゼリー無添加に比して有意差あり（Ｐ＜０．０５）

[0034]

[Experiment 4] For examination of the mechanism of enhancement of collagen production of the L-ascorbic acid by <examination of influence which it has on TGF-beta production of fibroblast by royal jelly and/or L-ascorbic acid 2-glucoside> royal jelly, The following methods considered the influence of royal jelly and/or L-ascorbic acid 2-glucoside on production of TGF-beta to which reinforcing production of mRNA of collagen of fibroblast is reported.

** Measuring method TGF-beta of TGF-beta was measured in TGF-beta 1 Emax ImmunoAssay System (Promega sale).

** In measurement of the production amount of measurement TGF-beta of royal jelly and/or the TGF-beta production amount of the fibroblast under L-ascorbic acid 2-glucoside existence. The fibroblast of the hamster prepared by the same method as the experiment 1 was planted by the $1x10^4$ cell / well to the microplate (BEKUTON DEKINSON sale) 96 well, and it cultivated until the cell covered the whole bottom of the well. Under existence of the royal jelly which dissolved only the D-MEM culture medium which contained 10% (v/v) of FCS after removing a culture supernatant so that it might become this culture medium in ml and 200 microg /, or nonexistence, the culture medium which dissolved L-ascorbic acid 2-glucoside so that it might become in ml and 0.2 microg /by the weight conversion as L-ascorbic acid -- or, Each culture medium which dissolved only royal jelly so that it might become in ml and 200 microg /was added by 200microl / well, it cultivated for 24 hours, and the amount of TGF-beta

in a culture supernatant was measured.

[0035]Hamster newborn infant fibroblast was producing TGF-beta of about 300 pg (it is hereafter written as "pg".) / ml also under the nonexistence of L-ascorbic acid 2-glucoside and royal jelly. The relative value which sets the TGF-beta production amount of the fibroblast under the nonexistence of this L-ascorbic acid 2-glucoside and royal jelly to 100 shows the result of an experiment to 4. The TGF-beta production amount of fibroblast did not change in addition of 200 microg/ml royal jelly. When the culture medium which, on the other hand, dissolved L-ascorbic acid 2-glucoside so that it might become in ml and 0.2 microg /by weight conversion of L-ascorbic acid is added, It is in the tendency for TGF-beta production to be reinforced as compared with additive-free, and the production amount was intentionally reinforced by addition of 200 microg/ml royal jelly. Production enhancement of this TGF-beta is well correlated with the enhancement by the royal jelly of the collagen production by L-ascorbic acid 2-glucoside of the newborn infant fibroblast of a hamster shown in the experiment 3, It is shown that the system through enhancement of production of TGF-beta exists in one of the mechanisms in which royal jelly reinforces the collagen production by L-ascorbic acid.

[0036]

[Table 4]

L－アスコルビン酸２－グルコシド濃度（μｇ／ｍｌ）	ローヤルゼリー添加の有無（２００μｇ／ｍｌ）	ＴＧＦ－β産生量 *
0.0	無	100±9
	有	98±9
0.2	無	111±5
	有	132±12 **

*：相対値±標準偏差

**：同濃度のＬ－アスコルビン酸２－グルコシド区において、ローヤルゼリー無添加に比して有意差あり（Ｐ＜０．０５）

[0037]

[Experiment 5] Since it was checked that production of TGF-beta of the fibroblast by <examination of influence which it has on TGF-beta production of keratinocyte by royal jelly> L-ascorbic acid is reinforced with royal jelly, Also about the keratinocyte in the epidermis which adjoins the dermis in which fibroblast exists, influence was considered that there is **** possibility to fibroblast, and the following methods considered the influence of royal jelly and/or L-ascorbic acid 2-glucoside on production of the TGF-beta.

** In culture of the culture-medium keratinocyte for keratinocyte culture. an EPIRAIFU (Epilife) culture medium (a cascade biologic ink company.) U.S. : to Cascade Biologics Inc. and USA. 0.06mM Ca^{++} and the growth additive agent HKGS (last concentration: -- a 5microg/ml cow insulin.) 5 microg/ml Cow transferrin and 0.5microM Hydrocortisone, a 0.2% cow hypophysis extract (cascade biologic ink company make.) The U.S., 100 unit/ml penicillin (Meiji Seika

Kaisha, Ltd. sale), and the EPIRAIFU +HKGS culture medium that added streptomycin (Meiji Seika Kaisha, Ltd. sale) 100 microg/ml were used as a basal medium for keratinocytes.
** Epidermis was exfoliated and centrifugal separation recovered precipitation from the piece of the regions-of-back skin of 4 age-in-day hamster newborn infant who prepared like the preparation experiments 1 of hamster newborn infant keratinocyte after the fragment using scissors. To the collected precipitation, 20 units/ml of DNase(s) (sigma company sale) were added, and the standard MEM culture medium (Nissui Sale and the following write it as "S-MEM".) was quietly added after churning for 3 minutes at the room temperature to it, and also centrifugal separation recovered the cell after churning for 2 minutes to it, and it was suspended to it at S-MEM. Culture of the following cells was performed in the incubator of 37 ** and 5%(v/v) CO_2 concentration.

** The keratinocyte suspended to measurement S-MEM of the TGF-beta production enhancement by royal jelly, centrifuging, collecting cells and being re-suspended to the basal medium for keratinocytes -- a type IV collagen coat -- it planted by a $1x10^4$ cell /100microl / well to the microplate (made by BEKUTON DEKINSON) 96 well. Keratinocyte removes culture medium after adhering to the bottom of a well, and it beforehand, The same royal jelly as what was used for the basal medium for keratinocytes in the experiment 1, 500, 250, 125, 62.5, and the test liquid contained 31.3 microg/ml replaced respectively the royal jelly which carried out stage dilution twice and prepared the solution which dissolved so that it might become in ml and 1000 microg /, and it by the basal medium for keratinocytes. Test liquid was removed three days afterward, the test liquid containing the royal jelly of the same concentration was added, further, it cultivated for 24 hours and the amount of TGF-beta in the supernatant liquid was measured by TGF-beta 1 Emax ImmunoAssay System like the experiment 4. In L-ascorbic acid and a royal jelly additive-free case, the KERATONO site was producing about 70 pg(s)/ml TGF-beta. The result of an experiment is shown in Table 5 with the relative value which sets these L-ascorbic acid and the TGF-beta production amount in a royal jelly additive-free case to 100.
** The same period keratinocyte is cultivated on the same conditions as the experiment of measurement ** of the promotion of growth of the keratinocyte by royal jelly, Allama after continuing culture for three more days after that blue (alamer blue) (Thorek die AGONO Stig systems ink company make: TREK DIAGONOSTIC SYSTEMS INC.) -- 20microl / well, [add and] After holding at 37 ** for 3 hours, the fluoro scan II (FluoroskanII, lab systems (Labsystems) company make) was used, and fluorescence intensity was measured with excited wavelengths of 544 nm, and the fluorescence wavelength of 590 nm. The relative value which set to 100 the amount of keratinocytes (fluorescence intensity) cultivated by royal jelly additive-free shows a result table 6.
[0038]Royal jelly was 125 microg [more than]/ml concentration, production of TGF-beta of

keratinocyte was reinforced depending on the concentration, and especially the operation was remarkable more than in 500 microg/ml. This acts also on the keratinocyte in the epidermis which adjoins the dermis in which it not only reinforces production of TGF-beta, but royal jelly acts on fibroblast and fibroblast exists, By TGF-beta which reinforces production of TGF-beta and is produced from both cells as a result. Also when it is shown that production of collagen of fibroblast is reinforced and the collagen production enhancement agent of this invention is directly used for the skin, Since it acts on the keratinocyte of epidermis before royal jelly reaches fibroblast, it is shown that the effect is demonstrated validity and promptly. Although concrete data was not shown, ascorbic acid did not affect the TGF-beta production ability or growth irrespective of the existence of the existence of royal jelly to keratinocyte.

[0039]

[Table 5]

ローヤルゼリー濃度（μg／ml）	TGF－β産生量 *
0.0	100±13
31.3	110±18
62.5	113±28
125.0	197±18
250.0	223±20
500.0	532±162 **
1000.0	734±230 **

*：相対値±標準偏差

**：ローヤルゼリー無添加に比して　P＜0．05（有意差有り）

[0040]It was 62.5 micro more thang/ml, and royal jelly showed the growth promotion operation of the keratinocyte depending on concentration, and was remarkable more than in 250 micro especiallyg/ml. Royal jelly does not have cytotoxicity, thru/or this shows the low thing. the collagen production enhancement agent of this invention -- keratinocyte -- ****** -- by reinforcing the cornified layer of the skin shows having the operation which strengthens the blophylaxis ability which the skin has.

[0041]

[Table 6]

ローヤルゼリー添加量（μg／ml）	ケラチノサイトの増殖量 *
0.0	100±6
31.3	102±7
62.5	115±15
125.0	114±5
250.0	128±7 **
500.0	138±3 **
1000.0	149±10 **

*：相対値±標準偏差
**：ローヤルゼリー無添加に比して P＜0．05（有意差有り）

[0042]
[Experiment 6] The royal jelly and L-ascorbic acid used as the raw material of the collagen production enhancement agent of <safety test of collagen production enhancement agent> this invention, It was used widely by the health food field, cosmetic field, etc., and although it was needless to say, it examined using the mouse that the safety was high about the safety of the collagen production enhancement agent of this invention just to make sure. The sodium L-ascorbate used in the experiment 1 by the weight conversion as L-ascorbic acid. To the one weight section, the preparation which mixed and prepared non-heat-treating royal jelly or heat treatment royal jelly (100 **, heating during 30 minutes) 4 weight section similarly used in the experiment 1 was diluted with deionized water of the amount of actual size, and was made into the preparation for an examination. Deionized water was used as a contrast article. Each on five males (average weight of 25 g, Charles River Japan Sale) of the DDY mouse of five weeks old. Per weight of 1 kg of a mouse, repetitive administration of an examination preparation and the contrast article was carried out for 30 days in taking orally so that it might be set to 10 g (it is considered as a collagen production enhancement agent, and they are 5 g/kg), and change of weight and observation of appearance were performed. The preparation for an examination was prepared in consideration of the stability of ascorbic acid just before administration every day.
[0043]Weight increased like the mouse of a control group and the health condition of the mouse of the group of a gap to administer orally to the collagen production enhancement agent which consists of sodium L-ascorbate, non-heat-treating royal jelly, or heat-treatment royal jelly was also good. Therefore, the safety of the collagen production enhancement agent of this invention containing L-ascorbic acid and royal jelly was judged to be a high thing from this experimental result.
[0044]Hereafter, although this invention is explained in more detail based on an example, this invention is not limited to these examples.
[0045]
[Work example 1]The ingredient below a <collagen production enhancement agent> was

uniformly mixed by the following combination, and the collagen production enhancement agent was prepared. This article was diluted with the culture medium for [of that] each of activity measurement according to the method of the experiment 1, the experiment 4, and the experiment 5, and it checked having the promotion of growth of having collagen production and a production operation of TGF-beta, and keratinocyte.
Sodium L-ascorbate 1.0 weight section Non-heat-treating royal jelly of use in the experiment 1 20.0 weight sections[0046]This article is a collagen production enhancement agent which shows the operation which reinforces the collagen production by L-ascorbic acid, gives grace to the skin, and takes effect to the aging prevention of the skin and which can use simple and shows higher efficacy. Since a moderate acid taste shows good taste, this article can be used also as the health food used daily, being not only useful but a TGF-beta production enhancement agent, or a KERACHINOSAI growth accelerator. As for this article, it is also free to also take in in taking orally as it is and to dissolve in water, other drinks, etc. and to take in. It is also free to blend with a food for specified dietary use, a food with health claims, cosmetics, quasi drugs, drugs, feed, a feed, pet food, general merchandise, etc., and to give these a collagen production operation, TGF-beta production potentiation, and/or a keratinocyte growth promotion operation.
[0047]
[Work example 2]After mixing the ingredient below <health food> uniformly by the following combination, this collagen production enhancement agent was used after reduced pressure drying, the grinder was used under ordinary temperature overnight, and the powdered collagen production enhancement agent was prepared. This article was diluted with D-MEM containing 10% (v/v) of FCS, the enhancement activity of collagen production of this article was measured according to the experiment 1, and it checked that the activity concerned was demonstrated.
L-ascorbic acid 2-glucoside (the Hayashibara Co., Ltd. biochemistry research institute sale) 1.0 weight sections Non-heat-treating royal jelly of use in the experiment 1 10.0 weight sections Hydrated crystal trehalose (a trade name "TOREHA", the Hayashibara Co., Ltd. trading company sale)
This agent may be adjusted to pH which is 38.0 weight sections add sodium hydroxide further if needed and corresponding to the use
[0048]This article is a collagen production enhancement agent which continues and shows collagen production potentiation and which can use simple and shows higher efficacy. This article is useful as health food daily used from excelling in mothball stability, without mellow sweet taste and a moderate acid taste not only showing good taste, but browning. This article can be advantageously used not only for Homo sapiens but for cultured animals, such as a fish as the ingestion for animals, such as livestock and a pet, or a constituent for intubation administration, a shrimp, and a crab, by mixing as direct or a bait.

[0049]To this collagen production enhancement agent, sucrose fatty acid ester was added so that it might become 1 % of the weight, and it fabricated to about 300 mg [per dose] tablet at it using the tableting machine. This article is a collagen production enhancement agent convenient also for a stable cellular phone for a long period of time with an easy ingestion which continues and shows collagen production potentiation and which shows higher efficacy.
[0050]
[Work example 3]Reduced pressure drying of the ingredient below <health food> was uniformly carried out after mixing by the following combination, and the paste state collagen production enhancement agent was prepared. It checked after preparation that the collagen production enhancement agent concerned was stabilized and collagen production potentiation was shown.
Sodium L-ascorbate 1.5 weight sections L-ascorbic acid 1.0 weight section The non-heat-treating royal jelly of use was heat-treated for 30 minutes at 70 ** by the experiment 1. Heat-treatment royal jelly 20.0 weight sections Sugar transition rutin (a trade name "alphaG rutin", the Hayashibara Co., Ltd. trading company sale)
1.0 weight sections Anhydrous crystal maltitol 1.5 weight sections[0051]This article is a collagen production enhancement agent which is stabilized and shows collagen production potentiation and which can use simple and shows higher efficacy. Since it not only has the effect excellent in normalization and aging prevention of skin structure, but [since this article is shown / collagen production potentiation /,] it shows good taste by mellow sweet taste and a moderate acid taste, it is useful as health food used daily.
[0052]
[Work example 4]<Ice cream> whipped cream (about 46 % of the weight of fats-and-oils contents) 18 weight section, Dissolve the mixture of powdered-skim-milk 7 weight section, whole milk 51 weight section, sugar 13 weight section, the amount part of pullulan duplexs, and Cyamoposis Gum 1 weight section, and after holding for 30 minutes and sterilizing at 70 **, with a homogenizer, carry out emulsification dispersion and it ranks second, After having quenched even at 3 thru/or 4 **, having added collagen production enhancement agent 5 weight sections obtained by the method of Example 2 to this, mixing further and riping overnight, it was made to freeze in a freezer and ice cream was obtained.
[0053]This article is ice cream which shows collagen production potentiation, gives grace to the skin, and takes effect to the aging prevention of the skin while showing moderate sweet taste and decent flavor.
[0054]
[Work example 5]
<Fruit jelly> sugar 14.0 weight sections Hydrated crystal trehalose 2.0 weight sections Gelatin 2.5 weight sections Grapefruit juice 32.0 weight sections Water 43.5 weight section The non-

heat-treating royal jelly of use in the experiment 1. Heat-treatment royal jelly heat-treated for 30 minutes at 70 ** 4.0 weight sections L-ascorbic acid 2.0 weight-section sugar, It heated at 95 **, trehalose, gelatin, and water were added, after the dissolution, grapefruit juice was added and it mixed, and further, after performing sterilization at 80 ** for 30 minutes, L-ascorbic acid and heat-treatment royal jelly were added, it cooled, and fruit jelly was prepared.
[0055]This article is fruit jelly in which collagen production potentiation is shown and which prevents aging of the skin and takes effect to maintenance and improvement of cosmetics and health while showing moderate sweet taste and a smooth texture.
[0056]
[Work example 6]<Health drink> anhydrous crystal malt sugar 500 weight section, collagen production enhancement agent 100 weight sections of Example 2, Powdered egg yellow 190 weight section, powdered-skim-milk 200 weight section, sodium chloride 4.4 weight section, Potassium chloride 1.85 weight section, magnesium sulfate 4 weight section, thiamin 0.01 weight section, The compound which consists of vitamin-E acetate 0.6 weight section and nicotinamide 0.04 weight section, and sugar transition hesperidin ("alphaG hesperidin PS", TOYO SUGAR REFINING CO., LTD. sale) 0.02 weight section was prepared. This compound 25 weight section was uniformly distributed and dissolved to purified water 150 weight section, and it enclosed 200g at a time with the brown glass bottle.
[0057]Since collagen production potentiation is made to maintain and also it is supplemented with the nutrient, this article can be advantageously used as a health drink aiming at cosmetics, health, etc. This article can be advantageously used also as the ingestion for animals, such as not only Homo sapiens but livestock, a pet, etc., or a constituent for intubation administration.
[0058]
[Work example 7]Add the collagen production enhancement agent which states compound ** to compound ** stated to the <skin external use cream> following below further in accordance with a conventional method after mixing and cooling at 30 ** or less, addition and, and with a potassium hydrate. pH was prepared to the acescence, it emulsified with the homogenizer, and skin external use cream was manufactured.
<compound **> monostearin acid polyoxyethylene glycerin 2.0 weight sections Self-emulsification type glyceryl monostearate 5.0 weight sections Behenic acid eicosanyl 1.0 weight section Liquid paraffin 1.9 weight sections Tori octanoic acid trimethylolpropane . 10.0 weight sections <Mixture **> 1,3-butylene glycol . 5.0 weight sections Sodium lactate liquid 10.0 weight sections . ginseng radix extract 1.5 weight sections Methyl parahydroxybenzoate . 0.1 weight sections Hyaluronate sodium 0.1 weight section Glycyrrhiza extract 0.5 weight section Purified water 62.4 weight sections <Collagen production enhancement agent> L-ascorbic acid 2-glucoside (the Hayashibara Co., Ltd. biochemistry research institute sale)

concern of critical side effects and also it has TGF-beta production potentiation and a keratinocyte growth promotion operation, animals including Homo sapiens can use it simple and comfortably for maintenance and improvement of the aging prevention of the skin, cosmetics, and health. Using as various constituents, such as foodstuffs, a drink, a food for specified dietary use, a food with health claims, cosmetics, quasi drugs, drugs, feed, a feed, pet food, and general merchandise, can also carry out advantageously the collagen production enhancement agent of this invention which has the above features by blending with other ingredients.

[0063]This invention is an invention which does so a operation effect also with remarkable ****. It is the invention meaningful [truly] which carries out a great contribution to the field.

[Translation done.]

2.0 weight sections Non-heat-treating royal jelly used in the experiment 1 3.0 weight sections, in addition this cream add a potassium hydrate if needed, and are still better also as weak alkaline cream.

[0059]Since not only making collagen production of the skin reinforce and maintain but this cream promotes growth of KERACHINOSAI by operation of reinforcing TGF-beta production of fibroblast or keratinocyte, Since maintain the freshness of the skin and a beam and sag are improved, and it is effective also in small JIWA wrinkles, and it excels in the preventive effect of aging and the outstanding moistness is shown, it is useful as basic cosmetics.

[0060]

[Work example 8]After mixing uniformly the ingredient below a <TGF-beta production enhancement agent> by the following combination, this TGF-beta production enhancement agent was used after neglect, the grinder was used under ordinary temperature overnight, and the powdered TGF-beta production enhancement agent was prepared. It diluted with the keratinocyte culture culture medium which uses this article in the experiment 5, the enhancement activity of TGF-beta production of this article and keratinocyte growth promotion activity were measured according to the experiment 5, and it checked that the activity concerned was demonstrated.

non-heat-treating royal jelly of use in the experiment 1 1.0 weight section Anhydrous crystal malt sugar (a trade name "fine TOSU", the Hayashibara Co., Ltd. trading company sale) 49.0 weight sections[0061]This article shows the operation which promotes the operation which carries out production enhancement and its growth of TGF-beta to keratinocyte, As a result, since collagen production of fibroblast is reinforced, it is a TGF-beta production enhancement agent which gives grace to the skin and takes effect to the aging prevention of the skin and which can use simple and shows higher efficacy. Since this article shows good taste by a moderate acid taste, it is as useful as the health food used daily. As for this article, it is also free to also take in in taking orally as it is and to dissolve in water, other drinks, etc. and to take in. It is also free to blend with a food for specified dietary use, a food with health claims, cosmetics, quasi drugs, drugs, feed, a feed, pet food, general merchandise, etc., and to give these collagen production potentiation, TGF-beta production potentiation, and/or a keratinocyte growth promotion operation.

[0062]

[Effect of the Invention]As opposed to the animal in which the collagen production enhancement agent in which this invention contains L-ascorbic acid and royal jelly contains Homo sapiens as explained above, The operation which reinforces the collagen production by the original remarkable L-ascorbic acid of non-heat-treating royal jelly is shown, and also it is completely based on the original knowledge of excelling in the durability of the operation concerned. Since the collagen production enhancement agent concerned does not have